



**COMPARATIVE EFFECTIVENESS AND OPTIONAL PERIOD  
OF THE FLOTATION METHOD USING NaCl, ZnSO<sub>4</sub> AND  
MgSO<sub>4</sub> FOR THE DIAGNOSTIC OF SOIL-TRANSMITTED  
HELMINTHS**

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Approval Page Of Research Report

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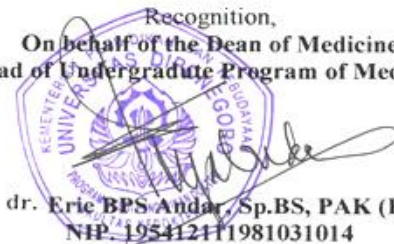
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## COMPARATIVE EFFECTIVENESS AND OPTIONAL PERIOD OF THE FLOTATION METHOD USING NaCl, MgSO<sub>4</sub> AND ZnSO<sub>4</sub> FOR THE DIAGNOSTIC OF SOIL TRANSMITTED HELMINTHS

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### ABSTRACT

**Background.** Factors that affect the effectiveness of flotation method in *Soil-transmitted helminths* diagnostic are flotation solution and optional period. Further research is required to determine the optional period that gives the best effectiveness from three flotation solutions, which are NaCl, MgSO<sub>4</sub>, and ZnSO<sub>4</sub>.

**Aim.** To compare the effectiveness of NaCl, MgSO<sub>4</sub> and ZnSO<sub>4</sub> as flotation solution in flotation method and the best optional period of each floating solution determined by counting number of eggs.

**Methods.** This research was quasi-experimental analytic with two independent variable. Independent variables 1 were flotation solutions (saturated NaCl, MgSO<sub>4</sub> and ZnSO<sub>4</sub>). Independent variables 2 were optional period (15,30,45,60 and 70 minutes). Each combination of treatments performed on 8 samples.

**Results.** There were significant differences between different of optional periods in one solution. The same optional period for each solution (saturated NaCl, saturated MgSO<sub>4</sub> and saturated ZnSO<sub>4</sub>) were not gives significant differences. The difference considered significant when the p value < 0.05. The most number of eggs found in NaCl was at 60 minutes, ZnSO<sub>4</sub> at 45 minutes, and MgSO<sub>4</sub> at 45 minutes).

**Conclusion.** The optimum of optional period for each solution were: saturated NaCl (60 minutes), ZnSO<sub>4</sub> (45 minutes), and MgSO<sub>4</sub> (45 minutes). Flotation method using ZnSO<sub>4</sub> as solution with 45 minutes as optional period considered as the most effective method based on number eggs found and time. In other hand, the effectiveness of the flotation method obtained either with NaCl, MgSO<sub>4</sub> and ZnSO<sub>4</sub> in all level of optional period were same.

**Keywords:** *flotation method, soil transmitted helminths, parasite diagnostic*

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# PERBANDINGAN EFEKTIVITAS DAN PERIODE OPSIONAL METODE FLOTASI MENGGUNAKAN NaCl, MgSO<sub>4</sub> DAN ZnSO<sub>4</sub> UNTUK DIAGNOSIS CACING TANAH

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## ABSTRAK

**Latar belakang.** Faktor-faktor yang mempengaruhi efektivitas pemeriksaan metode flotasi dalam diagnosis cacing tanah yaitu jenis larutan flotasi dan periode opsional. Penelitian lebih lanjut diperlukan untuk menentukan periode opsional yang memberikan hasil terbaik dari tiga larutan flotasi yaitu larutan NaCl jenuh, MgSO<sub>4</sub> jenuh, dan ZnSO<sub>4</sub> jenuh.

**Tujuan.** Untuk membandingkan efektivitas NaCl, MgSO<sub>4</sub> dan ZnSO<sub>4</sub> sebagai larutan pengapung dalam metode flotasi dan periode opsional terbaik untuk setiap larutan yang digambarkan dengan menghitung jumlah telur.

**Metode.** Penelitian ini merupakan studi analitik quasi-eksperimental dengan dua variabel bebas. Variabel bebas 1 adalah larutan flotasi (NaCl, MgSO<sub>4</sub> dan ZnSO<sub>4</sub>). Variabel bebas 2 adalah periode opsional (15,30,45,60 dan 70 menit). Setiap kombinasi perlakuan dilakukan pada 8 sampel.

**Hasil.** Terdapat perbedaan signifikan antara periode opsional berbeda dalam 1 larutan. Pada periode opsional yang sama untuk semua larutan (NaCl, MgSO<sub>4</sub> dan ZnSO<sub>4</sub>) tidak terdapat perbedaan signifikan. Perbedaan bermakna signifikan bila nilai  $p < 0.05$ . Jumlah telur terbanyak ditemukan pada NaCl menit ke 60, ZnSO<sub>4</sub> menit ke 45, dan MgSO<sub>4</sub> menit ke 45.

**Simpulan.** Periode opsional paling optimal untuk masing-masing larutan yaitu: NaCl (menit ke 60), ZnSO<sub>4</sub> (menit ke 45) and MgSO<sub>4</sub> (menit ke 45). Metode flotasi menggunakan ZnSO<sub>4</sub> jenuh sebagai larutan dengan 45 menit sebagai periode opsional memberikan hasil yang paling efektif berdasarkan pada jumlah telur yang ditemukan dan waktu. Di sisi lain, efektivitas metode flotasi yang diperoleh baik dengan larutan NaCl jenuh, MgSO<sub>4</sub> jenuh dan ZnSO<sub>4</sub> jenuh dalam semua tingkat periode opsional adalah sama.

**Kata kunci :** *metode flotasi, cacing tanah, diagnosis parasit*

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## INTRODUCTION

In general, the diagnosis of worm infection can be made clinically and epidemiologically. Clinical diagnosis of worms disease, especially hookworm (*Ancylostoma duodenale* and *Necator americanus*) can not give the right diagnosis because of hookworm not provide clear clinical symptoms, thus to assist the diagnosis required laboratory examination.<sup>1</sup> On direct examination, severe infection is easily to diagnose, while light infection can be examined by sedimentation method and parasite culture.<sup>1</sup> Examination of soil-transmitted helminths can be done by finding the worm eggs or adult worms in the stool examination.<sup>2</sup> Nowadays the most widely used technique in the operational diagnosis of parasitic either within the scope of the clinical and epidemiological survey was flotation method.<sup>2</sup>

Flotation technique showed high sensitivity as a diagnostic method of soil-transmitted helminths with a light infection (low infection rate). Therefore it's widely used as a definitive diagnosis in hospital and the scope of epidemiological surveys. Besides, this technique is quite complex and expensive due to the use of centrifugation but still the best method among the other methods.<sup>3</sup>

In general, the effectiveness of stool flotation examination is affected by the type of flotation solution, specific gravity of eggs and solution, flotation time (flotation period), and the homogeneity of the solution after the centrifugation process.<sup>4</sup>

The problem is many of laboratories doesn't have standard operational procedure (SOP) of flotation method. In other hand, this SOP is needed by the laboratorian to measure the most efficiency of time and diagnostic method of Soil-Transmitted helminth infection. This research tries to solve that problems.

This study aims to compare the effectiveness of NaCl, MgSO<sub>4</sub> and ZnSO<sub>4</sub> as flotation solution in flotation method and the best optional period for each floating solution in relation to the effectiveness which is drawing by counting number of eggs. This research has not been conducted before, thus this researcher propose to do this research. The results of this study are expected to become a

reference of standard operational procedure for the diagnosis of parasites in both clinical laboratories also for further research.

## METHODS

This research was quasi-experimental analytic with two independent variable. Independent variables 1 were flotation solutions (saturated NaCl, MgSO<sub>4</sub> and ZnSO<sub>4</sub>) . Independent variables 2 were optional period (15,30,45,60 and 70 minutes). Each combination of treatments performed on 8 samples.

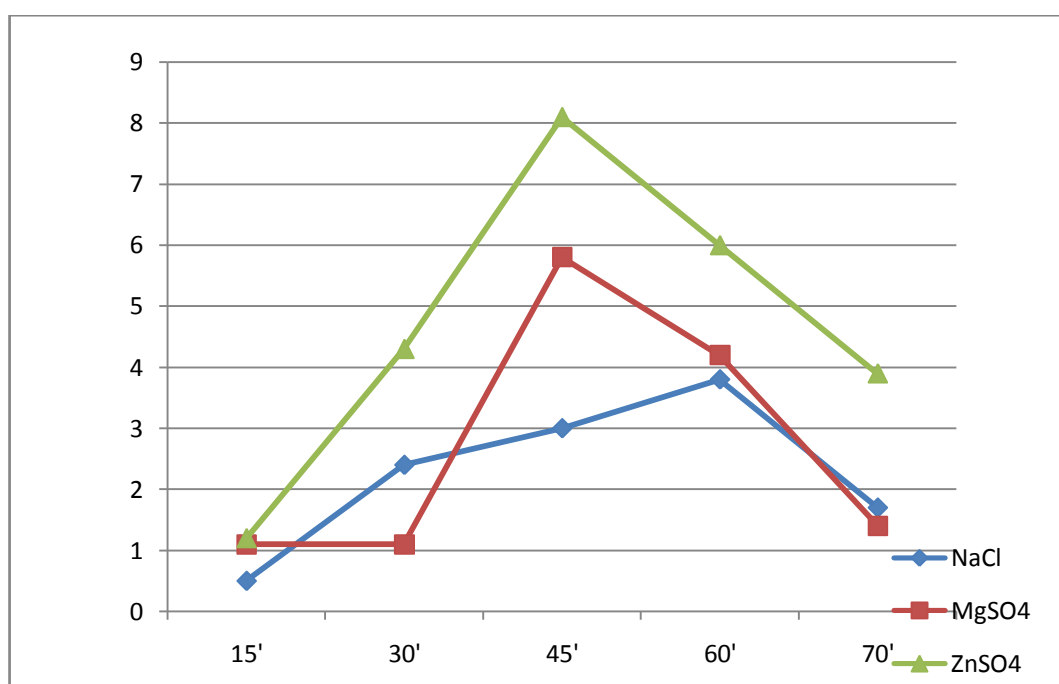
## RESULTS

Total positive samples used by researcher were 9 . Microscopic examination performed at 10x magnification to count the number of worm eggs and 40x magnification to see the morphology of the eggs. The number of eggs per gram (EGP) determined by counting the total number of eggs found in the entire field of view of the microscope divided by total grams of stool samples. The counting and microscopic observations was done twice by 2 different people in order to reduce the bias in counting. The result (Table 1) shows the total number of soil-transmitted helminths eggs found in sample with treatment groups.

**Table 1. Eggs Counting Number for each treatment groups**

No .	Code Of Sam ple	NaCl					MgSO <sub>4</sub>					ZnSO <sub>4</sub>				
		15	30	45	60	70	15	30	45	60	70	15	30	45	60	70
1	P/3	0	1	0	1	0	0	0	1	1	1	0	1	1	1	0
2	P/9	0	8	12	13	6	0	0	10	5	2	5	7	17	15	5
3	P/12	0	0	0	2	1	1	1	1	1	2	0	0	1	2	0
4	P/21	0	4	3	3	0	0	0	4	2	0	0	4	5	4	0
5	S/4	0	0	3	6	0	0	1	9	3	4	0	3	11	9	9
6	M/2	0	1	1	1	1	0	1	6	3	2	0	2	7	3	4
7	M/9	0	1	3	2	2	0	2	4	2	0	1	1	5	2	2
8	M/3	0	0	0	1	0	1	2	2	3	0	0	1	2	0	0

9	1 M/3 6	5	7	5	5	5	8	3	15	18	2	5	20	24	18	15
<b>Total</b>		<b>5</b>	<b>22</b>	<b>27</b>	<b>34</b>	<b>15</b>	<b>10</b>	<b>10</b>	<b>52</b>	<b>38</b>	<b>13</b>	<b>11</b>	<b>39</b>	<b>73</b>	<b>54</b>	<b>35</b>
<b>Average</b>		<b>0.</b>	<b>2.</b>	<b>3.</b>	<b>3.</b>	<b>1.</b>	<b>1.</b>	<b>1.</b>	<b>5.</b>	<b>4.</b>	<b>1.</b>	<b>1.</b>	<b>4.</b>	<b>8.</b>	<b>6.</b>	<b>3.</b>
		<b>5</b>	<b>4</b>	<b>0</b>	<b>8</b>	<b>7</b>	<b>1</b>	<b>1</b>	<b>8</b>	<b>2</b>	<b>4</b>	<b>2</b>	<b>3</b>	<b>1</b>	<b>0</b>	<b>9</b>



Regarding the optional period, the results were different for each solution, which are saturated NaCl solution, saturated MgSO<sub>4</sub> and saturated ZnSO<sub>4</sub>. With saturated NaCl, the effectiveness increased at 30 minutes to 60 minutes, then dropped continuously until the 70 minutes. With saturated ZnSO<sub>4</sub>, effectiveness increased at 30 minutes to 45 minutes, then dropped from 45 minutes to 70 minutes. With saturated MgSO<sub>4</sub>, the effectiveness increased at 30 minutes to 45 minutes, and then dropped at 45 minutes up to 70 minutes. At variance test, the difference was quite significant ( $p < 0.05$ )

Regarding the optional period, the results (Table 2 & Table 3) were different for each solution, which are saturated NaCl solution, saturated MgSO<sub>4</sub> and saturated ZnSO<sub>4</sub> saturated. With saturated NaCl, the effectiveness increased at 30 minutes to 60 minutes, then dropped continuously until the 70 minutes. With saturated ZnSO<sub>4</sub>, effectiveness increased at 30 minutes to 45 minutes, then dropped from 45 minutes to 70 minutes. With saturated MgSO<sub>4</sub>, the effectiveness increased at 30 minutes to 45 minutes, and then dropped at 45 minutes up to 70 minutes. At variance test, the difference was quite significant ( $p < 0.05$ ).

**Table 2. Friedman Test Results**

<b>Solution</b>	<b>15'</b>	<b>30'</b>	<b>45'</b>	<b>60'</b>	<b>70'</b>	<b>p</b>
NaCl	0,56	±2,44	±3,00	±3,78	±1,67	± 0,003*
	1,667	3,127	3,808	3,898	2,291	
MgSO <sub>4</sub>	1,11	±1,11	±5,78	±4,22	±1,44	± 0,001*
	2,619	1,054	4,738	5,310	1,333	
ZnSO <sub>4</sub>	1,22	±4,33	±8,11	±6,00	±3,89	± 0,000*
	2,167	6,245	7,897	6,516	5,183	

**Table 3. Wilcoxon Test Results**

<b>Solution</b>	<b>30'</b>	<b>45'</b>	<b>60'</b>	<b>70'</b>
NaCl				
15'	0,026*	0,039*	0,011*	0,066
30'	—	0,399	0,201	0,167
45'		—	0,084	0,102



60'			–	0,026*
<hr/>				
MgSO <sub>4</sub>				
15'	0,492	0,012*	0,011*	0,394
30'	–	0,018*	0,017*	0,618
45'		–	0,149	0,017*
60'			–	0,048*
<hr/>				
ZnSO <sub>4</sub>				
15'	0,018*	0,008*	0,012*	0,068
30'	–	0,011*	0,201	0,622
45'		–	0,020*	0,008*
60'			–	0,058
<hr/>				

Regarding the optional period, the same optional period in Table 4 for each solution ( saturated NaCl, saturated MgSO<sub>4</sub> and saturated ZnSO<sub>4</sub>) were not gives different results. At 15 minutes for each treatment groups of NaCl, ZnSO<sub>4</sub> and MgSO<sub>4</sub> p value was 0.524. At 30 minutes, p-value was 0.329, at 45 minutes p-value was 0.147, at 60 minutes p-value was 0.848, and at 70 minutes the p value was 0.754. The difference was not significant when the p value > 0.05.

**Table 4. Different test multivariate non pairedKruskal Wallis Test**

Time	Kruskal Wallis (p)
15'	0,524
30'	0,329
45'	0,147
60'	0,848
70'	0,754

## DISCUSSION

The use of  $\text{ZnSO}_4$  saturated as solution in the flotation method as for diagnostic soil-transmitted helminths is a new thing. Other conventional flotation solution: saturated NaCl, glucose and  $\text{MgSO}_4$  less effective for the detection of intestinal parasites because it can not float *Ascaris infertile* eggs, *Taenia spp* eggs and species Trematodes. The use of  $\text{ZnSO}_4$  as flotation solution seems to have pretty good prospects, in this study the saturated  $\text{ZnSO}_4$  can float infertile eggs of *Ascaris*, *Trichuris trichiura* eggs, and larvae *Strongyloides* perfectly. This can be happened because the saturated  $\text{ZnSO}_4$  solution used 1,270 specific gravity while specific gravity of infertile eggs of *Ascaris* and *Trichuris trichiura* eggs. average of 1.100 to 1.200. However, in this study there are still weaknesses, which is the condition of the sample. Samples used should contain species other intestinal parasites not only soil-transmitted helminths, that can represent three groups of intestinal parasites based on the average size of specific gravity.

Protozoan species with an average specific gravity under 1,100, species Nematodes and Cestodes by specific gravity on average from 1.100 to 1.200, as well as species Trematodes with specific gravity on average above 1,200. While the sample in this study only contains three soil-transmitted helminths species, while the Protozoa and Trematodes were not represented. Therefore, to obtain a more representative result, it is necessary to do research with more complete sample conditions (representing the three groups of intestinal parasites).

It can be concluded from the data, the effectiveness of the flotation method obtained either with saturated NaCl, saturated  $\text{MgSO}_4$  and saturated  $\text{ZnSO}_4$  in all level of optional period were same.

The weakness of this study was less references that discuss the influence of the flotation solution and the optional period with specific gravity of helminths eggs.

## **CONCLUSION**

The optimum of optional period for each solution were: saturated NaCl (60 minutes), ZnSO<sub>4</sub> (45 minutes), and MgSO<sub>4</sub> (45 minutes). Flotation method using ZnSO<sub>4</sub> as solution with 45 minutes as optional period considered as the most effective method based on number eggs found and time. In other hand, the effectiveness of the flotation method obtained either with NaCl, MgSO<sub>4</sub> and ZnSO<sub>4</sub> in all level of optional period were same.

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